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Effect of Organic Matter Decomposition Level on Bacterial Species Diversity and Composition in Relationship to Pythium Damping-Off Severity

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Rhizosphere bacteria were isolated from root tip segments of cucumber seedlings grown in a suppressive, slightly decomposed light-colored peat mix, a conducive, more decomposed dark-colored peat mix, and a suppressive dark peat mix amended with composted hardwood bark. The bacteria were identified by a gas chromatographic fatty acid methyl ester analysis. The total number of taxa recovered from a single root tip segment ranged from 9 to 18. No single taxon predominated on all root tip segments harvested from any of the mixes. The highest relative population density reached by a given taxon on any root tip segment was 45%. Hill's first and second diversity numbers, the modified Hill's ratio, and Hurlbert's rarefaction method, which were used as measures of species diversity, indicated that the organic matter decomposition level of the potting mixes did not affect bacterial species diversity. Bray-Curtis polar ordination and Dice resemblance functions, however, indicated that the organic matter decomposition level of a mix significantly influenced the composition of bacterial species in the rhizosphere. *Pseudomonas* spp. and other taxa capable of inducing suppression of pythium damping-off predominated in the suppressive mixes. These organisms were absent from the conducive mix, in which *Arthrobacter* and *Bacillus* spp. predominated. Although effective bacterial biocontrol agents were isolated from both the suppressive mixes and the conducive mix, the majority were isolated from the less decomposed suppressive mixes. Finally, the efficacy of strains was significantly greater in the slightly decomposed light peat mix than in the decomposed dark peat mix. Natural disease suppression within these mixes was associated with the organic matter decomposition level and the bacterial species compositions of the mixes.

A vast amount of information concerning the microbiology of native peatlands (lowmoor and highmoor bogs and fens) has been published since the pioneering efforts of Waksman and Purvis (55), Waksman and Stevens (56, 57), and Timonin (53). Relatively little is known, however, about the microbiology and microbial community ecology of sphagnum peat once it is harvested and processed (12). Even less is known about the microbiological factors that contribute to suppression of plant diseases in peat-based potting mixes.

Until recently, potting mixes prepared with peat as the sole source of organic matter were considered conducive to diseases caused by soilborne plant-pathogenic fungi, such as *Pythium* spp. (21). The addition of various types of mature composts to peat mixes may render them suppressive by increasing the level of microbial activity and inducing microbiostasis against the exogenously nutrient-dependent propagules of *Pythium* spp. (7, 8, 35). Disease suppression in these compost-amended mixes is due to the activity of many microorganisms. The microorganisms that are able to grow at high rates in the presence of both high and low nutrient concentrations (facultative oligotrophs) appear to be most important (50). General suppression, as described by Cook and Baker (11), best explains this mechanism of biological control of damping-off caused by *Pythium* spp. in compost-amended substrates (7, 8, 35).

During the last decade, the first reports of natural disease suppression in peat-based growing media were published (51, 59). These studies revealed that slightly decomposed light-colored batches of Finnish sphagnum peat harvested from the surfaces of bogs may suppress damping-off caused by various soilborne plant-pathogenic fungi, including *Pythium* spp. (51, 59). The suppressive effect of these peat batches is transitory but may persist for 7 weeks after planting. Subsequently, it was shown that Canadian sphagnum peats also vary in suppressiveness to pythium damping-off (24, 31). Inbar et al. (24) showed that the organic matter decomposition levels of the peats were related to the ability of the peats to induce suppression. Boehm and Hoitink (4) found that levels of root rot and populations of *Pythium ultimum* were highest in potting mixes prepared with a decomposed dark-colored peat and lower in mixes prepared with a slightly decomposed light-colored peat or a dark-colored peat amended with mature composted pine bark. Populations of *Pythium ultimum* and the occurrence of root rot were suppressed as long as the level of microbial activity, measured by the rate of hydrolysis of fluorescein diacetate, was maintained above a minimal threshold value of 3.2 μg of fluorescein min^{-1} g (dry weight) of mix^{-1}. Boehm and Hoitink concluded that both the organic matter decomposition level and the microflora in the potting mix contributed to sustained disease suppression.

In this study, we examined relationships among organic matter decomposition level, diversity and composition of rhizosphere bacterial communities, and suppressiveness to pythium damping-off. In addition, the abilities of individual bacterial strains to induce suppression of pythium damp-

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ing-off in potting mixes that differed in organic matter decomposition level were evaluated. This report is one of few (1, 3, 18, 41, 49) attempts to apply ecological statistical procedures, such as species diversity indices, rarefaction procedures, and polar ordination, to microbial ecosystems.

MATERIALS AND METHODS

Preparation of potting mixes. Three potting mixes differing in organic matter decomposition level and level of suppression of pythium damping-off were used in this study. Two mixes were prepared with Canadian sphagnum peat (Premier Peat Moss, Ltd., Rivière-du-Loup, Québec, Canada) and perlite as described previously (4). One of these mixes was prepared with a conducive, dark-colored, decomposed peat, classified as H_4 on the von Post decomposition scale (40) (referred to below as dark peat), and the other was prepared with a suppressive, light-colored, slightly decomposed (H_2) peat (referred to below as light peat). Both mixes were prepared by blending peat and coarse horticultural grade perlite (1:1, vol/vol) with (per liter) 7.1 g of $CaCO_3$ (particle diameter, <0.15 mm), 1.1 g of superphosphate, 1.1 g of KNO_3, and 1.1 g of gypsum. The third mix, a suppressive, composted hardwood bark-amended mix, referred to below as the compost-amended dark peat mix, was prepared by blending mature composted hardwood bark with conducive, dark-colored (H_4) sphagnum peat and coarse horticultural grade perlite (5:2:3, vol/vol/vol), as described by Chen et al. (8). Water was added to all mixes during blending (3 min) in a concrete mixer to bring the moisture level to 50% (wt/wt). The mixes were then stored for 14 days at 24°C. The air capacity of each mix was at least 15% (vol/vol) in a 10-cm-tall pot. The percolation rates were more than 2 cm/min, and the pH ranged from 5.5 to 6.2.

Preparation of inocula. *Pythium ultimum* 211, which originally was isolated from poinsettias (47), was used in this study. An inoculum was prepared as described previously (8) by using Ko and Hora's chopped potato soil medium (27).

Isolation, enumeration, and identification of bacteria. Bacteria were isolated from root tips of cucumber seedlings grown in each of the mixes, as described previously (29). The potting mixes were placed in plastic bags, amended with a slow-release fertilizer (Osmocote, N-P-K content of 14-14-14; 21 g/liter of mix; Grace-Sierra Chemical Co., Milpitas, Calif.), and shaken vigorously to ensure uniform distribution of the fertilizer. The mixes were then distributed into disposable styrofoam pots (400 ml per pot), and four cucumber (*Cucumis sativus* L. cv. Straight Eight) seeds (germination rate, 90%) were planted in each pot. The pots were irrigated daily and incubated for 8 days in a growth chamber at 20°C with 16 h of illumination (225 microeinsteins m^{-2} s^{-1}) per day. At harvest time, the seedlings were removed from each pot, shaken gently to remove excess potting mix, and then rinsed in 9.0 ml of sterile phosphate buffer containing (per liter of distilled water) 7 g of K_2HPO_4, 3 g of KH_2PO_4, and 0.2 g of $MgSO_4 \cdot 7H_2O$. A 1-cm root tip segment from the primary root was removed aseptically from a randomly selected seedling from each pot and comminuted in 100 μl of phosphate buffer with a Ten Broeck tissue homogenizer. The resulting suspension was serially diluted in sterilized phosphate buffer and plated in triplicate on 0.1× trypticase soy broth agar (TSBA) (BBL Microbiology Systems, Cockeysville, Md.). The mean number of CFU per root tip segment was determined after 4 days of incubation at 25°C. Next, approximately 70 colonies were nondiscriminately selected and streaked onto 0.1× TSBA. For root tip segments from which extremely small populations were recovered, all of the colonies on the lowest dilution plate were used. Bacterial strains were purified by repetitive streaking on 0.1× TSBA and were identified by performing a gas chromatographic fatty acid methyl ester analysis with a model HP5898A Microbial Identification System apparatus equipped with version 3.5 of the Aerobic Library (Microbial ID, Inc., Newark, Del.) according to the procedures specified by the manufacturer. The number of strains lost during subculturing was recorded. Strains with a similarity index of ≥0.5 were assigned to a species, strains with a similarity index of <0.5 and ≥0.1 were assigned to a genus, and strains with a similarity index of <0.1 were assigned a group (GC similarity group) on the basis of the similarity of the fatty acid profiles. Pure cultures were stored at −70°C in a 15% (vol/vol) sterile glycerol-water solution (46).

The relative population density of bacterial taxa on a root tip segment was calculated as follows: $100n_i/N$, where n_i is the number of strains assigned to the i^{th} taxon and N is the total number of strains on the root tip segment. The experiment was performed three times for each mix.

Efficacy of bacterial strains as biocontrol agents. Preliminary screening of all bacterial strains was performed in an autoclaved (1 h, 121°C) light peat mix infested with *Pythium ultimum*. Potting mixes were placed into plastic bags, amended with slow-release fertilizer, and infested with 0.5 g of *Pythium ultimum* soil inoculum per liter as described previously (4, 8). The bags were shaken vigorously to ensure uniform distribution of the inoculum and fertilizer. The infested mix was distributed into five disposable styrofoam pots (400 ml per pot) with perforated bases to ensure adequate drainage. Prior to planting, bacterial strains were cultured for 48 h in 50 ml of full-strength trypticase soy broth at 25°C with shaking (90 rpm), recovered by centrifugation for 10 min at 10,000 × *g*, and resuspended in phosphate buffer to a density of approximately 10^8 CFU/ml (verified by dilution plating). Approximately 50 cucumber seeds were added to the suspension, which was then mixed vigorously for 1 min. Eight treated seeds were planted 1 cm deep in each pot (five pots per treatment) by using sterile forceps. Controls consisting of cucumber seeds soaked in phosphate buffer and planted in both autoclaved and natural (nonautoclaved) light peat mix with and without the addition of *Pythium ultimum* were included in each bioassay. The pots were placed randomly in a growth chamber and incubated as described above. Disease severity was rated 10 days after planting according to the following scale: 1, symptomless; 2, emerged but wilted, chlorotic, or with visible lesions on the hypocotyl; 3, postemergent damping-off; and 4, preemergent damping-off. The mean disease severity for eight seedlings per pot was calculated and represented one treatment replication; there were five replicates per treatment. Diseased seedlings and nongerminated seeds were surface sterilized in 1% (vol/vol) sodium hypochlorite for 30 s, rinsed twice in sterile distilled water, and placed on a semiselective sucrose asparagine medium amended with pentachloronitrobenzene, neomycin sulfate, and chloramphenicol for reisolation of the pathogen (42). Randomly selected hyphal tips were transferred to lima bean agar (Difco Laboratories, Detroit, Mich.), and Middleton's description of *Pythium ultimum* was used to verify the identity of the pathogen (38).

The strains that were most effective for inducing suppression of pythium damping-off (mean damping-off severity values, 1.0 to 2.0) were reevaluated at least once with *Pythium ultimum*-infested autoclaved light and dark peat mixes.

Experimental designs and statistical analyses. Completely randomized designs were used in all cucumber bioassays. Each treatment was replicated five times (eight seedlings per pot and five pots per treatment). A one-way analysis of variance was performed by using Minitab statistical software (Minitab Inc., State College, Pa.). Separations of means were based on the least-significant difference ($P = 0.05$) (14).

Bacterial species (or taxon) diversity, defined as both the number of species present (species richness) and the distribution of strains among those species (species evenness), was determined for each root tip segment harvested from cucumber seedlings grown in all three potting mixes. Species richness was assessed by using Hurlbert's (23) rarefaction method. This method was selected instead of other richness indices because it allows comparisons to be made between communities based on unequal sample sizes, as was the case for the root tip segments screened in this study. The expected number of species (taxa) $[E(S_n)]$ was calculated by using the following equation:

$$E(S_n) = \sum_{i=1}^{s} \left\{ 1 - \left[\binom{N - n_i}{n} \Big/ \binom{N}{n} \right] \right\}$$

where S_n is the number of species (taxa) at a sample size of n. $E(S_n)$ was calculated as the sum of the probabilities that each species in a sample of size n will be included in the sample (32). Rarefaction curves were generated by plotting $E(S_n)$ versus n for each root tip segment.

Hill's (20) first and second diversity numbers (N_1 and N_2, respectively) (43), were used as overall measures of species diversity. N_1, a measure of abundant taxa, was calculated with the following equation: $N_1 = H'$, where H' is Shannon's index. Shannon's index was estimated by using the following equation:

$$\hat{H} = -\sum_{i=1}^{s} \left[\left(\frac{n_i}{N} \right) \ln \left(\frac{n_i}{N} \right) \right]$$

N_2, a measure of very abundant taxa, was calculated as follows: $N_2 = 1/\lambda$, where λ is Simpson's index (45). Simpson's index was calculated as follows:

$$\lambda = \sum_{i=1}^{s} p_i^2,$$

where p_i is the proportional abundance of the i^{th} taxon (n_i/N).

The modified Hill's ratio (E_5) (2) was used to assess species evenness and was calculated as follows: $E_5 = (N_2 - 1)/(N_1 - 1)$. E_5 ranges from 0 to 1. This evenness index is equal to 1 when all individual taxa are equally represented and approaches 0 when a single taxon is dominant in a community. The computer programs used in these analyses were RAREFRAC.BAS and SPDIVRS.BAS (32).

The levels of similarity in bacterial species composition among root tip segments (i.e., sampling units [SUs]) were determined by using Dice similarity coefficients (resemblance functions) and polar ordination (17, 33). Dice similarity coefficients were calculated as follows: $2a/(2a + b + c)$, where a is the number of common taxa which occur in two SUs (X and Y), b is the number of unique taxa which occur in SU X but not in SU Y, and c is the number unique taxa which occur in SU Y but not in SU X. This resemblance function is equal to 0 at no similarity and approaches 1 as the SUs become more similar to one another in bacterial species composition (33). The polar ordination method of Bray and Curtis (5, 17, 33) was used to position SUs within a coordinate system such that the distances between SUs reflected their levels of similarity in bacterial species composition as well as their relationships to underlying environmental gradients, such as organic matter decomposition level. In this study, SUs were arranged on the basis of percentages of dissimilarity. The percentage of dissimilarity between two SUs was calculated as follows: 100(1 − Dice similarity index). The computer programs used for these analyses were SPASSOC.BAS, SUDIST.BAS, and PO.BAS (33).

RESULTS

Relative population densities of bacterial taxa on cucumber root tip segments. The mean bacterial population sizes per root tip segment were similar regardless of mix and ranged between 1.1×10^4 and 5.6×10^5 CFU per root tip segment. The number of unique bacterial taxa isolated on 0.1× TSBA from most root tip segments increased until about 60 strains had been isolated. Whenever possible, this number of isolates was selected as an end point for recovery of taxa.

The relative population densities of bacterial taxa isolated from cucumber root tip segments grown in the compost-amended dark peat, light peat, and dark peat mixes are shown in Tables 1 through 3. No single taxon predominated on all root tip segments recovered from any of the mixes. The highest relative population density that a given taxon reached on any root tip segment was 45%. The total number of taxa recovered from a single root tip segment ranged from 9 to 18.

Pseudomonas spp. and members of GC similarity groups 1 and 6 predominated on root tip segments harvested from the suppressive compost-amended dark peat (Table 1) and light peat (Table 2) mixes. In general, these taxa accounted for the majority of the bacterial strains isolated from these root tip segments. *Pseudomonas* spp. were not isolated from root tip segments of seedlings grown in the conducive dark peat mix (Table 3). In addition, members of GC similarity groups 1 and 6 occurred only incidentally on root tip segments harvested from seedlings grown in the conducive dark peat mix (Table 3). *Arthrobacter* spp. predominated on root tip segments harvested from seedlings grown in the conducive dark peat mix (Table 3). These taxa were absent from root tip segments harvested from either of the suppressive mixes (Tables 1 and 2). The relative population densities of *Bacillus* spp. were significantly ($P = 0.05$) higher on root tips harvested from the conducive dark peat mix (6.9 to 8.5%) than on root tips harvested from either of the suppressive mixes (0 to 3.5%).

With the exception of two root tip segments, the minimum number of isolations per root tip segment was 35. Therefore, this sample size was used to compare the abundance of taxa on root samples directly (25). The expected number of bacterial taxa at this sample size $[E(S_{35})]$ obtained from root tip segments harvested from the mixes ranged from 9 to 15 (Fig. 1). The N_1, N_2, and E_5 values for each SU are shown in Table 4. In general, the differences among the three SUs within each of the mixes were as great or greater than the differences observed between the three mixes. This indicated that potting mix type did not affect bacterial species diversity, as determined by either species richness (S_{35}), species evenness (E_5), or a combination of these factors (N_1, N_2).

TABLE 1. Relative population densities of rhizosphere bacteria isolated on 0.1× TSBA from three cucumber seedling root tip segments grown in compost-amended dark peat potting mix

Taxon[a]	Relative population density in[b]:		
	SU 1	SU 2	SU 3
Agrobacterium spp.	3.0		
Alteromonas spp.	4.5		
Azotobacter spp.		2.9	2.7
Bacillus spp.			2.7
Comamonas testosteronii	1.5		41.1
Comamonas spp.	1.5	2.9	
Corynebacterium spp.			2.7
Cytophaga spp.			2.7
Enterobacter spp.		1.4	
Hydrogenophaga spp.		7.2	
Klebsiella spp.		1.4	
Micrococcus roseus	1.5		
Pseudomonas aureofaciens	1.5		
Pseudomonas cepacia		1.4	
Pseudomonas facilis	34.8		
Pseudomonas gladioli		4.3	
Pseudomonas solanacearum	1.5	1.4	
Pseudomonas spp.	7.6	39.1	
Xanthomonas spp.			1.4
GC similarity group 1	1.5	18.8	1.4
GC similarity group 2	4.5		11.0
GC similarity group 3	3.0	4.3	
GC similarity group 6	30.3		11.0
GC similarity group 7	1.5		1.4
GC similarity group 8		1.4	
GC similarity group 10			5.5
GC similarity group 11			12.3
GC similarity group 12		2.9	
GC similarity group 13	1.5	4.3	
Lost[c]		5.8	4.1

[a] Strains were identified by a gas chromatographic fatty acid methyl ester analysis on the basis of similarity of phospholipid fatty acid profiles. Strains with a similarity index of ≥0.5 were assigned to a species; strains with a similarity index of <0.5 and ≥0.1 were assigned to a genus; and strains with a similarity index of <0.1 were assigned to a GC similarity group.

[b] The relative population density of bacterial taxa on a root tip segment was calculated as follows: $100n_i/N$, where n_i is the number of strains assigned to the i^{th} taxon and N is the total number of strains on the root tip segment.

[c] Represents strains lost upon subculturing.

TABLE 2. Relative population densities of rhizosphere bacteria isolated on 0.1× TSBA from three cucumber seedling root tip segments grown in slightly decomposed light peat potting mix

Taxon[a]	Relative population density in[b]:		
	SU 1	SU 2	SU 3
Agrobacterium spp.	2.8	1.7	
Alcaligenes spp.	4.2		6.9
Bacillus pabuli	1.4		
Bacillus polymyxa	1.4		3.5
Bacillus spp.		1.7	
Cytophaga johnsonae	1.4		
Cytophaga spp.	2.8		
Flavobacterium balustinum			3.5
Flavobacterium spp.		8.3	
Hydrogenophaga spp.	1.4		3.5
Methylobacterium spp.		1.7	
Phyllobacterium spp.	4.2	5.0	
Pseudomonas aureofaciens	1.4		
Pseudomonas facilis	1.4		
Pseudomonas fluorescens	9.9		
Pseudomonas syringae pv. tabaci	1.4		
Pseudomonas spp.	26.8	21.7	20.7
GC similarity group 1	15.5	31.9	13.8
GC similarity group 2	5.6	1.7	3.5
GC similarity group 4		1.7	
GC similarity group 6	1.4		20.7
GC similarity group 7	9.9	1.7	
GC similarity group 8	1.4	5.0	24.1
GC similarity group 9		1.7	
GC similarity group 11		1.7	
Lost[c]	5.6	15.0	

[a] Strains were identified by a gas chromatographic fatty acid methyl ester analysis on the basis of similarity of phospholipid fatty acid profiles. Strains with a similarity index of ≥0.5 were assigned to a species; strains with a similarity index of <0.5 and ≥0.1 were assigned to a genus; and strains with a similarity index of <0.1 were assigned to a GC similarity group.

[b] The relative population density of bacterial taxa on a root tip segment was calculated as follows: $100n_i/N$, where n_i is the number of strains assigned to the i^{th} taxon and N is the total number of strains on the root tip segment.

[c] Represents strains lost upon subculturing.

Dice similarity coefficients indicating the proportional levels of similarity between SUs are shown in Table 5. Both the Dice similarity coefficients (Table 5) and the Bray-Curtis polar ordination plot (Fig. 2) confirmed that the bacterial species compositions on root tip segments harvested from cucumber seedlings grown in the three mixes differed. In general, the Dice similarity coefficients were highest for SUs harvested from the same potting mix (Table 5). The mean Dice similarity coefficients for the compost-amended dark peat, light peat, and dark peat mixes were 0.31, 0.46, and 0.46, respectively. The lowest Dice similarity coefficients were obtained when we compared SUs harvested from the suppressive compost-amended dark peat or light peat mixes and SUs harvested from the conducive decomposed dark peat mix. For instance, the Dice similarity coefficient for SU 3 harvested from the light peat mix and SU 2 harvested from the dark peat mix was 0.11 (Table 5). The Bray-Curtis polar ordination plot (Fig. 2) confirmed that the bacterial species compositions of SUs harvested from cucumber seedlings grown in the suppressive and the conducive mixes differed. The suppressive light peat SUs clustered in the upper right corner of the plot, whereas the conducive dark peat SUs clustered in the middle on the left. The compost-amended dark peat mix SUs were more scattered.

Efficacy of bacterial taxa as biocontrol agents. Of 200 strains isolated from root tip segments of seedlings grown in the compost-amended dark peat mix, 45 (~23%) induced suppression (mean damping-off severity values, <2.0) of pythium damping-off of cucumber in the slightly decomposed light peat mix. Of 157 strains isolated from root tip segments of cucumber seedlings grown in the light peat mix, 16 (~10%) were effective. In contrast, only 2 of 154 strains (~1%) isolated from root tip segments of cucumber seedlings grown in the conducive dark peat mix were effective. The two effective strains isolated from the decomposed dark peat mix were placed into GC similarity group 1 on the basis of their fatty acid profiles.

Effective strains isolated from the compost-amended dark peat mix were identified as strains of *Agrobacterium* spp., *Comamonas testosteronii*, *Comamonas* spp., *Corynebacterium* spp., *Cytophaga* spp., *Enterobacter* spp., *Hydrogenophaga* spp., *Micrococcus roseus*, *Phyllobacterium* spp., *Pseudomonas fluorescens*, *Pseudomonas* spp., *Xanthomonas* spp., and GC similarity group 7.

Effective strains isolated from the light peat mix included strains of *Agrobacterium* spp., *Alcaligenes* spp., *Hydrogenophaga* spp., *Pseudomonas fluorescens*, *Pseudomonas*

TABLE 3. Relative population densities of rhizosphere bacteria isolated on 0.1× TSBA from three cucumber seedling root tip segments grown in decomposed dark peat potting mix

Taxon[a]	Relative population density in[b]:		
	SU 1	SU 2	SU 3
Alcaligenes xylosoxydans			3.6
Alcaligenes nicotianae	1.4	2.1	
Arthrobacter spp.	1.4	12.8	7.1
Bacillus pabuli		6.4	
Bacillus polymyxa	1.4		
Bacillus spp.	5.5	8.5	7.1
Cellulomonas spp.	1.4		
Escherichia fergusonii	8.2		7.1
Klebsiella planticola	11.0		
Kluyvera spp.	9.6		
Ochrobacterium spp.			3.6
Phyllobacterium spp.			35.7
Xanthomonas maltophilia		12.8	
Xanthomonas spp.		2.1	3.6
GC similarity group 1	1.4	2.1	
GC similarity group 3	45.2	2.1	
GC similarity group 5	4.1	31.9	14.3
GC similarity group 6	6.8		
GC similarity group 7			10.7
GC similarity group 9	1.4	2.1	
Lost[c]	1.4	17.0	7.1

[a] Strains were identified by a gas chromatographic fatty acid methyl ester analysis on the basis of similarity of phospholipid fatty acid profiles. Strains with a similarity index of ≥0.5 were assigned to a species; strains with a similarity index of <0.5 and ≥0.1 were assigned to a genus; and strains with a similarity index of <0.1 were assigned to a GC similarity group.

[b] The relative population density of bacterial taxa on a root tip segment was calculated as follows: $100n_i/N$, where n_i is the number of strains assigned to the i^{th} taxon and N is the total number of strains on the root tip segment.

[c] Represents strains lost upon subculturing.

spp., and GC similarity group 1. Two unidentified actinomycete strains were also isolated from the slightly decomposed light peat mix. These strains were highly effective (range of damping-off severity values, 1.2 to 1.5) for inducing suppression of pythium damping-off; however, they induced phytotoxicity in cucumbers under the test conditions used, and no further characterization of these strains was attempted.

Table 6 shows the results obtained when we compared 12 effective bacterial strains in autoclaved light and dark peat mixes infested with *Pythium ultimum*. Although many strains induced a significantly (P = 0.05) higher level of disease suppression in the dark peat mix than in the autoclaved infested controls, pythium damping-off severity was significantly (P = 0.05) lower in the slightly decomposed light peat mix than in the decomposed dark peat mix.

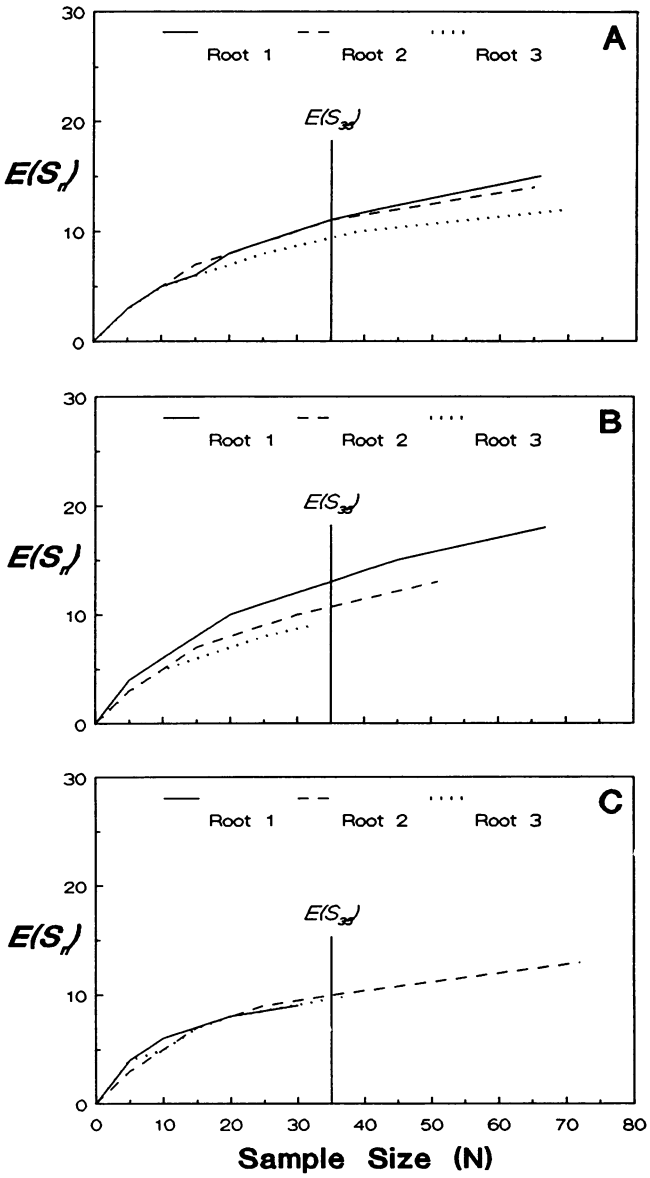

FIG. 1. Rarefaction curves for $E(S_n)$ for organisms isolated on 0.1× TSBA from three replicated root tip segments of cucumber seedlings grown in a compost-amended dark peat mix (A), a slightly decomposed light peat mix (B), and a more decomposed dark peat mix (C).

DISCUSSION

Biocontrol of pythium damping-off in the suppressive mixes undoubtedly was based on interactions of many different microorganisms. None of the bacterial biocontrol agents applied as a single treatment induced a level of disease suppression equal to that of the nonautoclaved control treatments infested with *Pythium ultimum* (Table 6). Many bacterial strains that exhibited activity against pythium damping-off were isolated from the suppressive mixes (23% of the strains isolated from the compost-amended dark peat mix and 10% of the strains isolated from the light peat mix). The conclusion of Chen et al. (9) and Mandelbaum and Hadar (35) that microbiostasis best explains the mechanism of suppression observed in compost-amended dark peat mixes is supported by our results. We propose that microbiostasis also occurs in the light peat and compost-amended dark peat mixes used in our study. Thus, the general suppression phenomenon, as postulated by Cook and Baker (11), best describes suppression of pythium damping-off in these suppressive mixes.

It is interesting that the bacterial species diversities (i.e., species richness and evenness), but not the species compositions, in the rhizospheres of cucumber seedlings harvested from the three mixes were very similar. The lack of differences in the total, but highly variable numbers of bacterial taxa isolated from root tip segments is consistent with previous reports on the microbial carrying capacity of rhizospheres (34). Differences in organic matter decomposition

TABLE 4. Species diversity indices for root tip segments harvested from cucumber seedlings grown in compost-amended dark peat mix, slightly decomposed light peat mix, and decomposed dark peat potting mix

Prepn[a]	Diversity indices		Evenness index E_5[d]
	N_1[b]	N_2[c]	
Compost-amended mix SU 1	4.65	1.93	0.62
Compost-amended mix SU 2	4.61	1.95	0.60
Compost-amended mix SU 3	5.50	1.87	0.63
Light peat mix SU 1	7.84	2.35	0.72
Light peat mix SU 2	4.81	1.89	0.68
Light peat mix SU 3	4.03	1.59	0.78
Dark peat mix SU 1	4.20	1.88	0.57
Dark peat mix SU 2	5.06	1.84	0.76
Dark peat mix SU 3	5.70	1.87	0.85

[a] Replicate root tip segments.
[b] N_1 is a measure of abundant taxa.
[c] N_2 is a measure of very abundant taxa.
[d] Evenness index E_5 is equal to 1 when all taxa are equally represented and approaches 0 when a single taxon is dominant in a community.

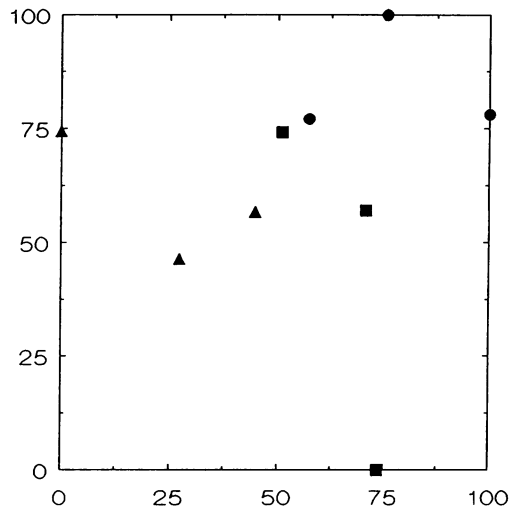

FIG. 2. Bray-Curtis polar ordination plot showing separation of SUs. Bacteria were harvested from root tip segments of cucumber seedlings grown in compost-amended dark peat mix (■), slightly decomposed light peat mix (●), and decomposed dark peat mix (▲). The axes represent percentages of dissimilarity.

level among the mixes, therefore, appeared to have little impact on the species diversity of the communities.

The relatively high level of similarity in bacterial species composition among SUs obtained from a given potting mix reflected homogeneity. The mean Dice similarity coefficients for the compost-amended dark peat, light peat, and dark peat mixes ranged from 0.31 to 0.46. On the other hand, the low means observed in comparisons between the light peat mix and the dark peat mix (mean Dice similarity coefficient, 0.18) or between the compost-amended dark peat mix and the dark peat mix (mean Dice similarity coefficient, 0.17) reflected heterogeneity. This suggested that the potting mix organic matter decomposition level influenced the bacterial taxon composition in the rhizosphere. The Bray-Curtis polar ordination plot (Fig. 2) supports this. In conclusion, SUs were separated on the basis of organic matter decomposition level and suppressiveness.

The bacterial taxa isolated from root tip segments of seedlings grown in the suppressive mixes were similar to the taxa described previously as biocontrol agents of soilborne plant pathogens, including *Pythium* spp. (16, 30, 50). Pseudomonads typically accounted for 25 to 45% of the total number of strains isolated from root tip segments of seedlings in the suppressive mixes. The presence of pleomorphic taxa, such as *Arthrobacter* spp. (relative population density range, 3 to 15%), and the lack of isolation of pseudomonads from root tip segments of seedlings grown in the decomposed dark peat mix revealed that the composition of bacterial taxa in the conducive habitat resembled that in mineralized soil fractions (22, 26, 54).

The relatively high population density of *Arthrobacter* spp. on root tip segments harvested from seedlings grown in the conducive dark peat mix is consistent with the observation of Martin (36), who noted that although *Arthrobacter* populations are low in native dark peat, *Arthrobacter* spp. could account for up to 30% of the total number of strains isolated from such peat after exposure to various chemical amendments. Furthermore, it has been shown that the bacterial microflora in native dark peat consists largely of anaerobic bacteria, pleomorphic forms (*Arthrobacter* and *Nocardia* spp.), and *Bacillus* spp. (10, 13, 15, 37, 44, 58). The predominance of *Bacillus* spp. in the more decomposed dark peat may be due to the preferential survival of spores under the anaerobic, low-pH conditions that prevail at that depth in

TABLE 5. SU comparison matrix of similarity coefficients (resemblance functions) for bacterial taxa isolated from root tips of cucumber seedlings grown in compost-amended dark peat mix, slightly decomposed light peat mix, and decomposed dark peat mix

Prepn	Dice similarity coefficient[a]							
	Compost-amended mix		Light peat mix			Dark peat mix		
	SU 2	SU 3	SU 1	SU 2	SU 3	SU 1	SU 2	SU 3
Compost-amended mix SU 1	0.41	0.37	0.49	0.25	0.33	0.21	0.15	0.24
Compost-amended mix SU 2		0.15	0.36	0.22	0.40	0.16	0.17	0.27
Compost-amended mix SU 3			0.35	0.27	0.30	0.08	0.00	0.29
Light peat mix SU 1				0.45	0.54	0.19	0.14	0.15
Light peat mix SU 2					0.38	0.23	0.26	0.27
Light peat mix SU 3						0.29	0.11	0.00
Dark peat mix SU 1							0.61	0.36
Dark peat mix SU 2								0.42

[a] Dice similarity coefficients were calculated as follows: $2a/(2a + b + c)$, where a is the number of common taxa which occur in two SUs (X and Y), b is the number of unique taxa which occur in SU X but not in SU Y, and c is the number unique taxa which occur in SU Y but not in SU X. This resemblance function is equal to 0 at no similarity and approaches 1 as the SUs become more similar to one another in bacterial species composition.

TABLE 6. Ability of selected bacterial strains to induce suppression of damping-off of cucumber in autoclaved light and dark peat mixes infested with *Pythium ultimum*

Taxon or prepn	Source[a]	Damping-off severity[b]	
		Light peat mix	Dark peat mix
Agrobacterium spp.	Light peat mix	1.4	3.4
Alcaligenes spp.	Light peat mix	1.8	3.1
Hydrogenophaga spp.	Light peat mix	1.4	2.8
Phyllobacterium spp.	Compost-amended mix	1.8	3.5
Pseudomonas fluorescens	Compost-amended mix	1.8	3.3
Pseudomonas fluorescens	Light peat mix	1.9	3.5
Pseudomonas fluorescens	Light peat mix	1.9	3.2
Pseudomonas spp.	Compost-amended mix	1.9	2.7
Pseudomonas spp.	Light peat mix	2.0	3.4
Pseudomonas spp.	Light peat mix	1.8	3.6
Pseudomonas spp.	Light peat mix	1.9	2.6
GC similarity group 1	Dark peat mix	1.6	3.1
Nonautoclaved control mix		1.1	1.1
Nonautoclaved infested mix		1.0	2.6
Autoclaved control mix		1.2	1.0
Autoclaved infested mix		3.7	3.6

[a] Bacterial strains were isolated from the rhizospheres of cucumber seedlings grown in compost-amended dark peat mix, light peat mix, and dark peat mix.

[b] Mean damping-off severity ratings for cucumber seedlings grown in autoclaved light peat and dark peat mixes infested with 0.5 g of a soil inoculum containing *Pythium ultimum* per liter ($n = 40$). The cucumber seedlings were rated 10 days after planting according to the following scale: 1, symptomless; 2, emerged but wilted, chlorotic, or with visible lesions on the hypocotyl; 3, postemergent damping-off; and 4, preemergent damping-off. The least significant difference ($P = 0.05$) was 0.7.

bogs from which the peat is harvested (48). Thus, the relatively high population density of *Bacillus* spp. isolated from the rhizosphere of cucumber seedlings in the dark peat mix (6.9 to 8.5%) compared with the population densities of *Bacillus* spp. in the suppressive light peat mix (which has a higher microbial carrying capacity) and the compost-amended dark peat mix (0 to 2.7%) is consistent with previous reports on the microbiology of native peatlands.

The predominance of gram-negative bacteria, such as *Pseudomonas* spp., in slightly decomposed light peat (analogous to the surface layer of bogs) and the predominance of *Bacillus* spp. and members of pleomorphic genera, such as *Arthrobacter* spp., in dark peat (analogous to the subsurface layers of bogs) are what one would expect to find if minimal changes in microbial species composition take place during the peat-harvesting process. As a result, the indigenous microbial community in the peat would be well positioned to exploit the mild biological vacuum created during mix preparation when the pH of the peat is raised from a range of 3.1 to 3.9 to a range of 5.5 to 6.2. On the other hand, this treatment also offers opportunities for the controlled introduction of biocontrol agents.

The *Arthrobacter* strains tested in this work were not effective as biocontrol agents. Although *Arthrobacter* strains that exhibit lytic activity against plant pathogens, including *Pythium aphanidermatum*, have been described previously (39), in this study their role in biological control in the rhizosphere appeared to be insignificant.

We did not attempt to enumerate and characterize oligotrophic rhizosphere bacteria. Sugimoto et al. (50) showed that obligate oligotrophs isolated from the rhizosphere of cucumber did not induce suppression of pythium damping-off when they were applied as seed treatments. These microorganisms, as well as other taxa that predominate in mineralized soil fractions (22, 26, 54) and in the rhizosphere of cucumber seedlings grown in compost-amended dark peat mixes (30, 50), could well play a role in suppression of the pathogen population. This aspect of disease suppression was not explored in this work because high rates of *Pythium* propagule death have not been encountered in these mixes (4, 9, 35). The oligotrophs could well be most active in the low-rate, long-term eradication of *Pythium* propagules observed in compost-amended potting mixes previously (8, 35).

The significantly ($P = 0.05$) greater efficacy of bacterial biocontrol agents in the slightly decomposed light peat mix than in the decomposed dark peat mix (Table 6) shows that the organic matter decomposition levels of the mixes had an impact on suppression of pythium damping-off. The enhanced efficacy of the bacterial biocontrol agents observed in the slightly decomposed light peat mix may be attributable to cellulase activity since several of these taxa produce this enzyme (55, 60). On the other hand, in the more decomposed and lower-cellulose-content dark peat mix (6, 15, 28, 40, 52, 56), in which substrate availability limits microbial activity (4), growth of these microorganisms and the potential for biological control depend more on the assimilation of rhizosphere deposition products (34).

During the formulation of a peat mix, the pH is raised from the pH of native peat (pH 3.1 to 3.9) to pH 5.5 to 6.2. This removes the inhibitory effect of peat on colonization by most soil bacteria. During the blending process, considerable fragmentation of peat "fibers" takes place, thus exposing fresh, previously unavailable surface area to the activity of decomposers. Heal et al. (19) offered a similar explanation for the stimulation of microbial activity in the surface layer of bogs as a result of soil fauna activity. In the slightly decomposed light peat and compost-amended dark peat mixes, the impact of this effect should be much greater than in the decomposed dark peat. The short burst of microbial activity observed previously in mixes prepared with slightly decomposed light peat and the greater activity in the compost-amended dark peat mix support this hypothesis (4). Sustained activity of the beneficial microflora in the light peat mix, therefore, depends on the availability of slow-release sources of nutrients for growth and activity. This property of peats needs to be explored further to characterize fully the general suppression phenomenon.

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REFERENCES

1. **Acea, M. J., and T. Carballas.** 1990. Principal components analysis of the soil microbial population of humid zone of Galicia (Spain). Soil Biol. Biochem. **22**:749–759.
2. **Alatalo, R. V.** 1981. Problems in the measurement of evenness in ecology. Oikos **37**:199–204.

3. **Atlas, R. M.** 1984. Diversity of microbial communities. Adv. Microb. Ecol. **7**:1–47.
4. **Boehm, M. J., and H. A. J. Hoitink.** 1992. Sustenance of microbial activity in potting mixes and its impact on severity of *Pythium* root rot of poinsettia. Phytopathology **82**:259–264.
5. **Bray, J. R., and J. T. Curtis.** 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. **27**: 325–349.
6. **Brown, A., S. P. Mathur, T. Kauri, and D. J. Kushner.** 1988. Measurement and significance of cellulose in peat soils. Can. J. Soil Sci. **68**:681–685.
7. **Chen, W., H. A. J. Hoitink, and L. Madden.** 1988. Microbial activity and biomass in container media for predicting suppressiveness to damping-off caused by *Pythium ultimum*. Phytopathology **78**:1447–1450.
8. **Chen, W., H. A. J. Hoitink, and A. F. Schmitthenner.** 1987. Factors affecting suppression of *Pythium* damping-off in container media amended with composts. Phytopathology **77**:755–760.
9. **Chen, W., H. A. J. Hoitink, A. F. Schmitthenner, and O. Tuovinen.** 1988. The role of microbial activity in suppression of damping-off caused by *Pythium ultimum*. Phytopathology **78**: 314–322.
10. **Collins, V. G., B. T. D'Sylva, and P. M. Latter.** 1978. Microbial populations in peat, p. 94–112. *In* O. W. Heal and D. F. Perkins (ed.), Production ecology of British moors and montane grasslands, vol. 27. Springer-Verlag, New York.
11. **Cook, R. J., and K. F. Baker.** 1983. The nature and practice of biological control of plant pathogens. American Phytopathological Society, St. Paul, Minn.
12. **Cormier, Y., A. Mériaux, and G. Brochu.** 1988. Effects of processing on peat moss microflora. Can. J. Microbiol. **34**:131–133.
13. **Dawid, W.** 1984. Myxobakterien in ungestörten Hochmooren des Hohen Venn (Hautes Fagnes, Belgien). Syst. Appl. Microbiol. **5**:555–563.
14. **Devore, J., and R. Peck.** 1986. Statistics: the exploration and analysis of data. West Publishing Co., New York.
15. **Dooley, M., and C. H. Dickinson.** 1970. The microbiology of cut-away peat. II. The ecology of fungi in certain habitats. Plant Soil **32**:454–467.
16. **Elad, Y., and I. Chet.** 1987. Possible role of competition for nutrients in biocontrol of *Pythium* damping-off. Phytopathology **77**:190–195.
17. **Gauch, H. G., Jr. (ed.).** 1982. Ordination, p. 109–172. *In* Multivariate analysis in community ecology. Cambridge University Press, New York.
18. **Gilbert, G. S., J. L. Parke, M. K. Clayton, and J. Handelsman.** 1993. Effects of an introduced bacterium on bacterial communities on roots. Ecology **74**:840–854.
19. **Heal, O. W., P. M. Latter, and G. Howson.** 1978. A study of the rates of decomposition of organic matter, p. 136–159. *In* O. W. Heal and D. F. Perkins (ed.), Production ecology of British moors and montane grasslands, vol. 27. Springer-Verlag, New York.
20. **Hill, M. O.** 1973. Diversity and evenness: a unifying notion and its consequences. Ecology **54**:427–432.
21. **Hoitink, H. A. J., and P. C. Fahy.** 1986. Basis for the control of soilborne plant pathogens with composts. Annu. Rev. Phytopathol. **24**:93–114.
22. **Holding, A. J.** 1960. The properties and classification of the predominant gram-negative bacteria occurring in soil. J. Appl. Bacteriol. **23**:515–525.
23. **Hurlbert, S. H.** 1971. The nonconcept of species diversity: a critique and alternative parameters. Ecology **52**:577–586.
24. **Inbar, Y., M. J. Boehm, and H. A. J. Hoitink.** 1991. Hydrolysis of fluorescein diacetate in sphagnum peat container media for predicting suppressiveness to damping-off caused by *Pythium ultimum*. Soil Biol. Biochem. **23**:479–483.
25. **James, F. C., and S. Rathburn.** 1981. Rarefaction, relative abundance, and diversity of avian communities. Auk **98**:785–800.
26. **Kanazawa, S., and Z. Filip.** 1986. Distribution of microorganisms, total biomass, and enzyme activities in different particles of brown soils. Ecology **12**:205–215.
27. **Ko, W., and F. K. Hora.** 1971. A selective medium for the quantitative determination of *Rhizoctonia solani* in soil. Phytopathology **61**:707–710.
28. **Kozlovskii, F. I.** 1958. Mikroflora pochv Karapuzskogo zaimishcha i ee izmenenie pri sel'skokhozyaistvennom osvoenii. Bulletin of Experimental Works and Scientific Research of the Ubinskaya Agricultural Experiment Station, no. 4. (In Russian.) Selkhozgiz Publishers, Moscow.
29. **Kwok, O. C. H., P. C. Fahy, H. A. J. Hoitink, and G. A. Kuter.** 1987. Interactions between bacteria and *Trichoderma hamatum* in suppression of *Rhizoctonia* damping-off in bark compost media. Phytopathology **77**:1206–1212.
30. **Kwok, O. C. H., H. A. J. Hoitink, and W. Chen.** 1987. Characterization of bacteria involved in suppression of *Pythium* damping-off. Phytopathology **77**:1707.
31. **Le Quéré, D., S. Gagné, and M. Caron.** 1990. Ability of sphagnum peat moss to suppress damping-off caused by *Pythium ultimum*. Can. J. Plant Pathol. **12**:335–336.
32. **Ludwig, J. A., and J. F. Reynolds.** 1988. Diversity indices, p. 85–103. *In* Statistical ecology: a primer on methods and computing. John Wiley and Sons, New York.
33. **Ludwig, J. A., and J. F. Reynolds.** 1988. Polar ordination, p. 211–222. *In* Statistical ecology: a primer on methods and computing. John Wiley and Sons, New York.
34. **Lynch, J. M., and J. M. Whipps.** 1990. Substrate flow in the rhizosphere. Plant Soil **129**:1–10.
35. **Mandelbaum, R., and Y. Hadar.** 1990. Effects of available carbon source on microbial activity and suppression of *Pythium aphanidermatum* in compost and peat container media. Phytopathology **80**:794–804.
36. **Martin, N. J.** 1971. Microbial activity in peat with reference to the availability and cycling of inorganic ions. Ph.D. thesis. University of Edinburgh, Edinburgh.
37. **Martin, N. J., J. Siwasin, and A. J. Holding.** 1982. The bacterial population of a blanket peat. J. Appl. Bacteriol. **53**:35–48.
38. **Middleton, J. T.** 1943. The taxonomy, host range and geographic distribution of the genus *Pythium*. Mem. Torry Bot. Club **20**:1–171.
39. **Mitchell, R., and R. Hurwitz.** 1965. Suppression of *Pythium deharyanum* by lytic rhizosphere bacteria. Phytopathology **55**: 156–158.
40. **Puustjärvi, V., and R. A. Robertson.** 1975. Physical and chemical properties, p. 23–28. *In* D. W. Robinson and J. G. D. Lamb (ed.), Peat in horticulture. Academic Press, Inc., New York.
41. **Rosswall, T., and E. Kvillner.** 1978. Principal components and factor analysis for the description of microbial populations. Adv. Microb. Ecol. **2**:1–48.
42. **Schmitthenner, A. F.** 1980. *Pythium* species: isolation, biology and identification, p. 33–36. *In* F. O. Larsen and B. G. Joyner (ed.), Advances in turfgrass pathology. Harcourt Brace Jovanich, New York.
43. **Shannon, C. E., and W. Weaver.** 1949. The mathematical theory of communication. University Illinois Press, Urbana.
44. **Shunk, I. C.** 1929. Microbiological activities in the soil of an upland bog in eastern North Carolina. Soil Sci. **27**:283–304.
45. **Simpson, E. H.** 1949. Measurement of diversity. Nature (London) **163**:688.
46. **Sleesman, J. P., and C. Leben.** 1978. Preserving phytopathogenic bacteria at −70°C or with silica gel. Plant Dis. Rep. **62**:910–913.
47. **Stephens, C. T., and C. C. Powell.** 1983. *Pythium* species causing damping-off of seedling bedding plants in Ohio greenhouses. Plant Dis. **66**:731–733.
48. **Stout, J. D.** 1971. Aspects of the microbiology and oxidation of Wicken Fen soil. Soil Biol. Biochem. **3**:9–25.
49. **Strom, P. F.** 1985. Effect of temperature on bacterial species diversity in thermophilic solid-waste composting. Appl. Environ. Microbiol. **50**:899–905.
50. **Sugimoto, E. E., H. A. J. Hoitink, and O. H. Tuovinen.** 1990. Enumeration of oligotrophic rhizosphere pseudomonads with diluted and selective media formulations. Biol. Fertil. Soils

9:226–230.
51. **Tahvonen, R.** 1982. The suppressiveness of Finnish light coloured sphagnum peat. J. Sci. Agric. Soc. Finl. **54:**345–356.
52. **Theander, O.** 1954. Studies on sphagnum peat. III. A quantitative study on the carbohydrate constituents of *Sphagnum* mosses and *Sphagnum* peat. Acta Chem. Scand. **8:**989–1000.
53. **Timonin, M. I.** 1935. The micro-organisms in profiles of certain virgin soils in Manitoba. Can. J. Res. **13:**32–46.
54. **Topping, L. E.** 1937. The predominant micro-organisms in soils. I. Description and classification of the organisms. Zentralbl. Bakteriol. **97:**289.
55. **Waksman, S. A., and E. R. Purvis.** 1932. The microbiological population of peat. Soil Sci. **34:**95–113.
56. **Waksman, S. A., and K. R. Stevens.** 1928. Contribution to the chemical composition of peat. II. Chemical composition of various peat profiles. Soil Sci. **26:**239–251.
57. **Waksman, S. A., and K. R. Stevens.** 1929. Contribution to the chemical composition of peat. V. The role microorganisms in peat formation and decomposition. Soil Sci. **28:**321–340.
58. **Wheatley, R. E., M. P. Greaves, and R. H. E. Inkson.** 1976. The aerobic bacterial flora of a raised bog. Soil Biol. Biochem. **8:**453–460.
59. **Wolffhechel, H.** 1988. The suppressiveness of sphagnum peat to *Pythium* spp. Acta Hortic. (The Hague) **221:**217–222.
60. **Zagural'skaya, L. M.** 1976. Micro-inhabitants of marsh peat soils in Tomsk Region, p. 54–87. *In* N. I. P'yavchenko (ed.), Interrelation of forest and bog. Amerind, New Delhi, India. (Translated from Russian by S. T. Shende.)